



TITLE:

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CITATION:

WATANABE, RYO. Pathology of Bone Sarcoma Induced by 20-Methylcholanthrene. 日本外科宝函 1966, 35(3): 437-461

ISSUE DATE:

1966-05-01

URL:

<http://hdl.handle.net/2433/207311>

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Pathology of Bone Sarcoma Induced by 20-Methylcholanthrene

by

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Received for Publication Feb. 22, 1966

I. INTRODUCTION

For the purpose of study on the biological mechanism responsible for the development of neoplasm, many trials have been made to produce experimental tumors in animals^{2,17,67)}. MARTLAND's observation on osteogenic sarcoma which developed in radium dial painters stimulated investigators to produce bone tumors in animals with radioactive substances. SCHÜRCH and UEHLINGER (1931) succeeded in producing osteogenic sarcoma in the rabbit by intramedullary administration of radium⁶⁰⁾. They also reported the induction of EWING's sarcoma by mesothorium which was injected in the bone marrow of the rabbit's femur⁶²⁾. SABIN et al. reported bone sarcoma induced by intravenous injection of radium chloride and mesothorium in rabbits⁵⁷⁾. Recently radioactive isotopes are also being used to produce experimental bone sarcoma^{33,68)}.

Since YAMAGIWA and ICHIKAWA (1915) reported the production of cancer by repeated painting of tar on the ear of a rabbit, many chemical carcinogens have been introduced in the field of tumor research^{8,16)}. KENNAWAY was responsible for determination of 3,4-benzpyrene as the active substance in carcinogenic pitch. WIELAND et al. synthesized the fully aromatic and powerfully carcinogenic hydrocarbon 20-methylcholanthrene^{23,36,42,43)}. Besides these powerful carcinogenic chemicals, various kinds of substances such as croton oil, o-aminoazotoluen, aromatic amine, quinine, and beryllium, and even glucose solution and distilled water are also supposed to be carcinogenic^{3,18,22,24,26,46,48,75)}.

Production of bone tumors with chemical carcinogen was first reported by ANARDI (1934)¹⁾. In his experiment osteochondroma was developed in the rabbit's ear by the administration of tar. BRUNSCHWIG and BISSELL observed the development of osteogenic sarcoma in mice by the injection of 3,4-benzpyrene into the bone marrow¹¹⁾. Since that time on, production of bone sarcoma in animals has been reported by BRUNSCHWIG¹²⁾, RUSH et al⁵⁶⁾, FRANSEEN et al¹⁹⁾, OBAYASHI⁴⁵⁾, MAKITA³⁹⁾, CHINO¹³⁾, and YAMADA⁷⁹⁾.

Transplantable bone sarcoma was also produced by virus injection into the bone marrow (FUJINAMI, ROUS, KATO, and BERG)^{6,20,21,29)}.

Simultaneous administration of radioactive phosphorus with 20-methylcholanthrene or 3,4-benzpyrene increased the rate of tumor production in rats (MAKITA).

Investigations of new bone formation in bone sarcoma has been performed by PHEMIS-

TER⁵³⁾, BRUNSWIG & HARMON⁹⁾, UNO⁷⁴⁾, and TORIYAMA et al.⁷¹⁾ However, detailed comments about new bone formation in bone sarcoma, to the present time, have not been made.

In this experiment the author attempted to examine the course of development of experimental bone sarcoma using 20-methylcholanthrene in rats. Furthermore, after transplantation of experimentally produced bone sarcoma into the bone marrow and beneath the periosteum, the process of new bone formation in bone sarcoma and metastasis was also examined.

II. THE EXPERIMENTAL PRODUCTION OF BONE SARCOMA IN RATS

MATERIALS AND METHOD

Fifty young rats of both sexes of the Gifu-Donryu strain, weighing 80-100 g, were employed for this experiment. 20-methylcholanthrene suspended in olive oil was used as the carcinogenic agent.

The lower end of the right femur was selected as the site of injection of the carcinogenic agent. Under nembutal anesthesia a needle was inserted into the lower end of the femur through the patellar ligament and intercondylar fossa, and 0.1 ml of the suspension containing 3 mg of 20-methylcholanthrene was injected into the medullary cavity of the femur (Fig. 1). The animals died or were sacrificed one to fifty-two weeks after the injection. All the animals were radiographed, subjected to necropsy and preserved in 10 per cent formalin solution. The femurs were decalcified, embedded in celloidin, sectioned and stained by hematoxylin and eosin and by phosphotungstic-acid-hematoxylin.

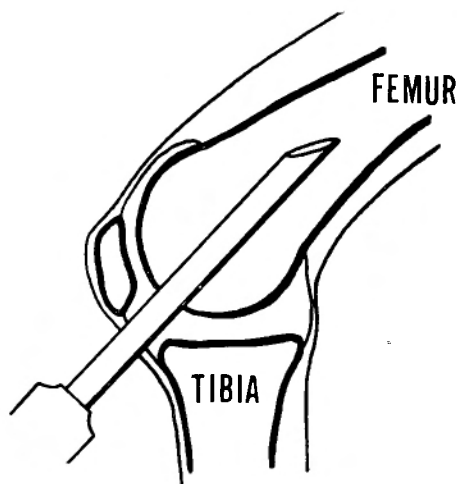


Fig. 1. Technique of injection of 20-methylcholanthrene into the marrow cavity of the femur.

RESULTS

Results of this experiment are summarized in Table 1, 2 and 3. Out of 50 rats, 17 died without evidence of tumor formation within 10 weeks after the injection and histologic examination revealed fibrosis of the marrow cavity of the femur. Palpable tumors were produced in the lower end of the femur in 11 of the 33 rats. The rate of tumor production was 33.3 per cent. Twenty-two weeks after the injection, the first tumor was found in Case 203 and the last tumor was detected in Case 601 forty-seven weeks after the injection. The average period of time needed for tumor production was 35 weeks after the injection of the carcinogenic agent. The tumor became palpable when it increased approximately one centimeter in diameter. Sudden growth occurred in Cases 102, 203, and 302.

Bone tumors which developed in this experiment included 10 fibrosarcomas and one

Table 1 Experimental Production of Bone Sarcoma by Intramedullary Injection of 20-Methylcholanthrene

Nos. of Rats	Invalid		Valid		Rate of Tumor Production (per cent)
	Accidental Death	Others	Development of Tumor	Negative	
50	17	0	11	22	33.3

Table 2 Findings in Cases of Bone Sarcoma Induced by 20-Methylcholanthrene

Case Number	Observation Period (weeks)	Roentgenographic Findings	Histologic Findings	Metastasis
101	30	Osteolytic	Fibrosarcoma	None
102	38	Osteolytic	Fibrosarcoma	None
203	22	Osteolytic	Fibrosarcoma	Pulmonary metastasis
204	43	Osteoplastic	Osteogenic sarcoma	None
207*	29	Osteolytic	Fibrosarcoma	None
301	31	Osteolytic	Fibrosarcoma	None
302	35	Osteolytic	Fibrosarcoma	None
304	42	Osteolytic	Fibrosarcoma	None
305	42	Osteolytic	Fibrosarcoma	None
601	47	Osteolytic	Fibrosarcoma	None
602	31	Osteolytic	Fibrosarcoma	None

* Successive transplantation was performed successfully.

Table 3 Roentgenographic Findings, Gross Pathology, and Microscopic Findings in Bone Sarcoma Induced by 20-Methylcholanthrene

Case Number	Roentgenographic Findings	Gross Pathology	Microscopic Findings
101, 102, 207, 301, 302, 304, 305, 601, and 602.	Shortening of the femur, thickening of the cortex, and cortical defects. No evidence of new bone formation in the tumor.	Smooth, reddish-gray, friable, and soft tumor, capsulated with thin fibrous membrane. Grayish-white central part with rubbery consistency. No evidence of metastasis.	Lack of osteogenesis, spindle-shaped stromal cells, atypism of cells and cell nuclei, mitotic division and sarcomatous giant cells.
203	Rarefaction of the lower part of the femur and the patella. Enlargement of the upper end of the tibia.	Capsulated, soft and fragile, reddish-gray tumor, with central necrosis. Positive pulmonary metastasis.	
204	New bone formation in the tumor tissue, enlarged femur, cortical defects in the shaft and condyles. Bulging and radiolucent patches in the upper end of the tibia. Narrowing of the joint space of the knee.	Large tumor with hard consistency. Cut surface of the tumor is partly grayish-white and hard and partly grayish-red and soft. No evidence of metastasis.	Spindle-shaped and highly anaplastic stromal cells, large hyperchromatic 'bizarre' nuclei, mitotic division, neoplastic osteoid, osseous tissue, and cartilage formation.

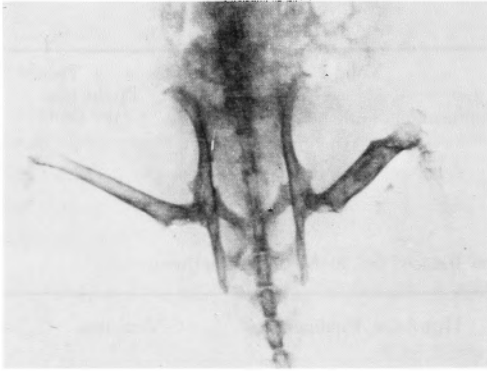


Fig. 2. Roentgenogram of the right femur in which bone sarcoma did not develop.

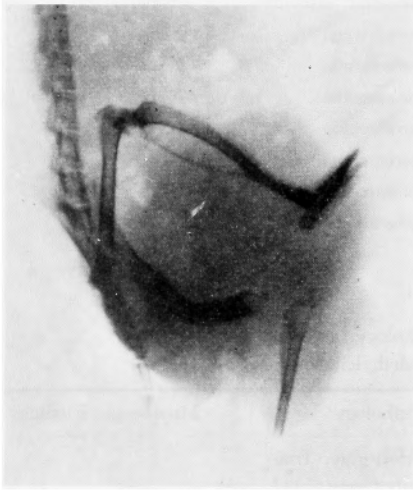


Fig. 3. Case 203. Radiolucent areas in the lower third of the femur suggesting development of a tumor of central origin.

and enlargement of the upper end of the tibia were found. These roentgenographic findings suggest development of a tumor of central origin. There was no evidence of a sunray-stream-like shadow or new bone formation in the tumor tissue (Fig. 3). In the remaining nine cases, except for Case 203, roentgenograms of the femur showed bone destruction of lesser degree.

Gross pathology :

Case 203. A tumor of $6.0 \times 6.0 \times 6.0$ cm developed in the right thigh. The skin was adherent to the tumor and necrotic area was found in the lateral aspect of the thigh. The lower end of the shaft and condyles of the femur were entirely occupied by tissue which was soft, fragile, reddish-gray in color, and was encapsulated by membranous tissue. Miliary white patches were found in the lung.

Case 207. A soft tumor was found in the right thigh. The tumor was reddish-

osteogenic sarcoma. Roentgenographic, gross pathologic and histologic findings of the bone in the cases in which tumors did not develop and in the cases in which sarcoma developed were as follows :

1. Cases in which the tumor did not develop.

Roentgenogram of the femur showed a marked decrease in the length, increase in width, and thickening of the cortex (Fig. 2). In most cases, the lower end of the femur was enlarged and irregularly outlined. In some cases, cortical defects of various size were found in the shaft and metaphysis, especially in the former. Occasionally periosteal new bone formation, probably due to the stimulation of 20-methylcholanthrene which flowed beneath the periosteum through VOLKNANN's canal from the marrow cavity, was observed. Microscopic examination showed marked fibrosis of the bone marrow and irregular thickening of the cortex and periosteum.

2. Cases of fibrosarcoma (Cases 101, 102, 203, 207, 301, 302, 304, 305, 601, and 602).

Roentgenographic findings :

Shortening of the length of the femur, thickening of the cortex, and cortical defects were common findings in these 10 cases. In Case 203, complete loss of the lower third of the femur, radiolucent area in the patella,

gray and friable and was encapsulated with a thin fibrous membrane. The central part of the tumor was grayish-white and of a rubbery consistency. No metastasis was found.

The remaining eight tumors, except for the two cases mentioned above, presented pathologic findings similar to those in Case 207. No evidence of metastasis was shown in all these cases.

Microscopic findings :

All 10 cases showed similar histologic characteristic features such as lack of osteogenesis, spindle-shaped stromal cells, atypical cells and cell nuclei, hyperchromatism of the nuclei, mitotic division, and sarcomatous giant cells (Figs. 4 and 5). The original bone was moderately destroyed by the growth of the tumor tissue. In Case 203, the lower third of the femur had dissolved and the condyles were entirely replaced by the tumor

tissue. At the lower end of the femur the periosteum was elevated by the tumor. This fact suggests that the tumor is of central origin. In Case 601, the original bone cortex was compressed by the tumor tissue which developed parosteally. These 10 cases were diagnosed as having fibrosarcomas.

3. Case of osteogenic sarcoma (Case 204).

Roentgenographic findings :

In this case (Fig. 6), the femur was considerably enlarged and, in addition, a cortical defect was found in the lower half of the shaft and the condyles. Moreover, irregular dense shadows suggesting new bone formation were seen in the interior of the affected bone. The upper end of the tibia likewise revealed bulging and radiolucent patches. The knee joint space was narrowed.

Gross pathology :

A large tumor, weighing 185 g, with hard consistency developed in the right thigh.

The overlying skin was easily removed from the tumor. Many dilated veins were found around the tumor. The cut surface of the tumor showed the mixed feature of hard grayish-white parts and soft grayish-red parts. The lower half of the femur and the upper third of the tibia was replaced with tumor tissue, while the articular cartilage seemed to be intact. No evidence of tumor metastasis was found.

Microscopic findings.

The tumor tissue exhibited osteogenesis of moderate degree. Within an unossified region the stromal cell was spindle-shaped and highly anaplastic. Most of the cells contained large hyperchromatic bizarre nuclei (Fig. 7). The original bone was markedly destroyed by the growth of the sarcomatous tissue and, in some regions, it was broken



Fig. 6. Case 204. Roentgenogram of the osteogenic sarcoma. Irregular dense shadows in the femur and in the tumor suggesting new bone formation.

into fragments. In the part in which new bone was formed, a large amount of intercellular collagenous material developed and trabeculae of osteoid and calcium deposits in the intercellular matrix were observed. These new bone consisted of loosely woven bone trabeculae interspersed with loose-meshed tumor cells which showed evidence of moderate malignancy, as shown in Figs. 8 and 9. In some areas of the tumor tissue, the cartilaginous tissue was seen to arise from the sarcomatous tissue as shown in Fig. 10.

From these findings, this case was diagnosed as having osteogenic sarcoma.

III. SUCCESSIVE SUBCUTANEOUS TRANSPLANTATION OF EXPERIMENTALLY PRODUCED FIBROSARCOMA OF BONE

MATERIAL AND METHOD

Each of eleven bone sarcomas obtained was implanted into the subcutaneous tissue of rats of the same strain. The fibrosarcoma induced in Case 207 was successfully taken. This tumor was of poor differentiation. The central part of this tumor showed rubbery consistency and its periphery was soft and friable. The area of rubbery consistency consisted of spindle-shaped fibroblasts arranged in whorled pattern and considerable amount of intercellular wavy and interlacing collagen fibers. At the periphery the tissue showed cellular atypism, excessive cellularity, lack of uniformity in the size of the cells, hyperchromatism of cell nuclei and mitotic division.

A piece of the tissue was excised from the peripheral soft part of the tumor, cut into small pieces and crushed by a simple grinder. The crushed tissue was then implanted into subcutaneous tissue of the rat of the same strain (Gifu-Donryu strain, weighing 80-100 g). All these procedures were performed aseptically.

RESULTS

Successive transplantation of sarcoma tissue into the subcutaneous tissue was carried out in 116 rats over sixteen generations and positive results were obtained in 100 cases. The rate of successful transplantation was 87.7 per cent. Metastasis to other organs has not occurred (Table 4).

The tumor-bearing rat died 28 to 75 days after the transplantation with an average of 42.1 days. One week after the transplantation the tumor became palpable. Four weeks after the transplantation, anemia, falling-off of hair, and loss of body weight occurred in the host animals. The tumor was covered with a thin membrane and was easily detached from the surrounding subcutaneous tissue. The tumor tissue was soft, fragile, and reddish-gray in color. Central softening was not uncommon. Local recurrence

Table 4 Results of Successive Subcutaneous Transplantation of Fibrosarcoma of Bone Induced by 20-Methylcholanthrene in Case 207.

Nos. of Rats	Invalid	Valid		Rate of Successful Transpl. (per cent)	Rate of Metast. (per cent)	Period 'till Death of the Animal after the Transplantation (days)	
		Takes	Neg.			Min.-Max.	Average
116	2	100	14	87.7	0	28-75	42.1

has not appeared after the removal of the tumor.

Microscopic findings of the tumor presented the cytologic pattern of the original tumor during the course of successive transplantations during sixteen generations for a period of one year.

IV. INTRAMEDULLARY TRANSPLANTATION OF EXPERIMENTALLY PRODUCED FIBROSARCOMA OF BONE

MATERIALS AND METHOD

The fibrosarcoma of bone which had been experimentally produced and successively transplanted into the subcutaneous tissue of rats was transferred into the bone marrow of the left tibia of rats of the same strain. A piece of the tumor tissue was excised, cut into small pieces, packed in a syringe with a needle of No. 17 gauge or into a glass-made trocar and implanted into the marrow cavity of the tibia through the patellar ligament and articular surface (Fig. 11).

RESULTS

The results of intramedullary transplantation of experimentally produced bone sarcoma are shown in Table 5. One hundred and six rats were employed in this experiment. Ten of them died by accident and seven escaped from the cage. In 61 rats tumor developed in the left tibia and 28 showed no evidence of take of the tumor implants. The rate of successful transplantation was 68.6 per cent. Metastases were found in various organs in 14 of

the 61 cases (23.1 per cent); in the lung in eleven cases, the liver in two, and the spleen in one. The rate of metastasis to the lung was 18.0 per cent. These results were compared with those of the experiments in which WALKER's carcinosarcoma 256 and HORIE's sarcoma are used (Table 6). The metastatic rate to the lung was higher in the cases of WALKER's carcinosarcoma than in the cases of methylcholanthrene bone sarcoma. Fur-

Table 5 Results of Intramedullary Transplantation of Fibrosarcoma of Bone Induced by 20-Methylcholanthrene in Case 207.

Nos. of Rats	Invalid	Valid		Rate of Successful Transpl. (per cent)	Rate of Metast. (per cent)	Period 'till Death of the Animal after the Transplantation (days)	
		Takes	Neg.			Min.-Max.	Average
106	17	61	28	68.6	23.1	14-70	35.6

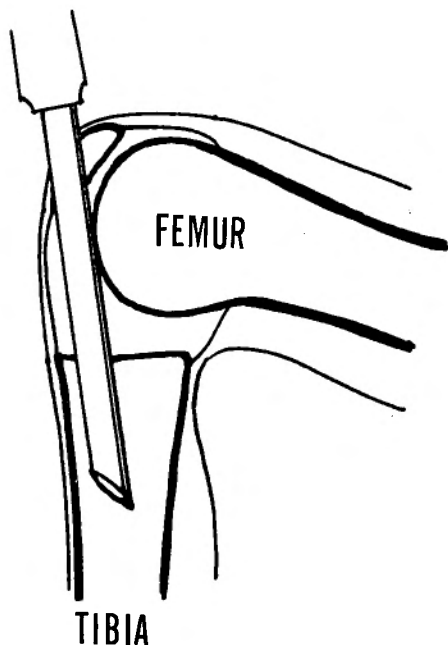


Fig. 11. Technique of intramedullary implantation of the tumor.

Table 6 Comparison of Results of Intramedullary Transplantation of Bone Sarcoma of Three Kinds.

	Nos. of Rats	Invalid	Valid		Rate of Successful Transpl. (per cent)	Rate of Metast. (per cent)
			Takes	Neg.		
Walker's carcino-sarcoma 256 (Kita, 1963)	135	10	98	27	78.4	83.7
Horie's sarcoma (Taniguchi, 1963)	70		30		42.8	23.3
20-Methylchol. Fibrosarcoma (Watanabe, 1966)	106	17	61	28	68.6	23.1

thermore, the successful transplantation rate was higher in cases of methylcholanthrene bone sarcoma than in cases of HORIE's bone sarcoma.

Development of the bone tumor in the tibia.

Local and general conditions of the host animals :



Fig. 12. Roentgenogram of the tibia taken two weeks after the implantation.

One week after the implantation the tibia did not show any palpable enlargement. Two weeks after the implantation swelling of the leg appeared. One week later, extensive swelling of the leg, loss of body weight, falling-off of hair, and anemia occurred in host animals. The animals died 14 to 70 days after the implantation with an average of 35.6 days.

Roentgenographic findings :

One week after the implantation, the tibia showed no evidence of bone destruction or new bone formation. Two weeks after the implantation thinning of cortex, radiolucent patches in the shaft of the tibia, and swelling of soft part were found as shown in Fig. 12. However,

destructive change did not seem to invade the epiphyseal cartilage.

Three weeks after the implantation, marked rarefaction of bone structure of the shaft, bulging of the cortex, honey-comb-like structure of the shaft and the metaphysis, and pathologic fracture were common findings in all animals. Nevertheless, destructive change never extended beyond the epiphyseal cartilage. The knee joint seemed to remain intact during the course of experiment. Figs 13, 14 and 15 show the roentgenologic findings of the tibia mentioned above.

Gross pathology :

The pathologic changes were first observed two weeks after the implantation. The bone marrow cavity was found to contain a tumor mass. Three weeks after the implantation, autopsy revealed that the upper two thirds of the tibia showed spindle-shaped enlargement and the bone marrow of the shaft and the metaphysis was thoroughly replaced with the tumor tissue.

Microscopic findings :

One week after the implantation, histologic study showed development of the tumor

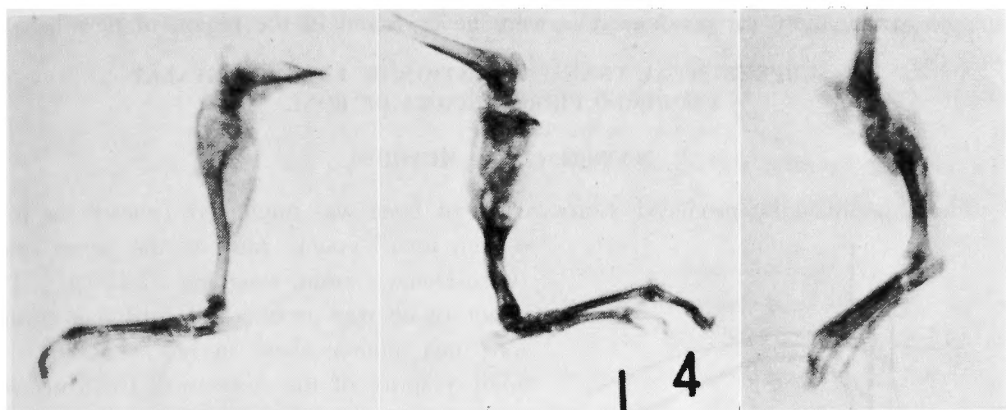


Fig. 13. Bulging of the cortex. **Fig. 14.** Honey-comb-like structure. **Fig. 15.** Pathologic fracture.
Figs. 13, 14 and 15. Roentgenograms of the tibia taken three weeks after the implantation.

tissue in the bone marrow. The tumor cells were found both in VOLKMANN's canal and beneath the periosteum. There was no evidence of new bone formation in the tumor tissue so far as it was confined within the bone marrow.

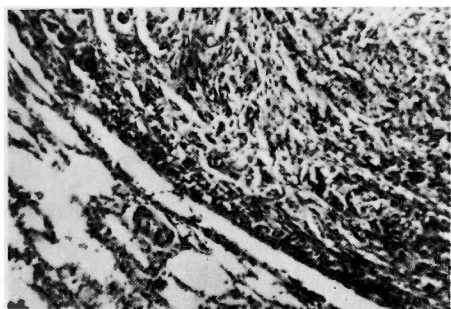


Fig. 18. Photomicrograph of the metastatic lesion in the lung. Hematoxylin and eosin, $\times 100$.

Histologic examination two weeks after the implantation revealed that the tumor tissue filled up the marrow cavity of the shaft and the metaphysis and that thinning of the cortex due to resorbing of the trabeculae was in progress. The other part of the specimen showed marked destruction of the cortical bone and development of tumor tissue beneath the periosteum (Fig. 16). Moreover, formation of minute new bones were seen in the tumor tissue. Metastases of tumor were found in the lung, liver, and spleen (Fig. 17). These metastatic lesions grew in

the same cytological pattern as the original tumor (Fig. 18).

Three weeks after the implantation the bone cortex was resorbed and the tumor tissue was found to be extended into the surrounding soft parts as shown in Fig. 19. At the periphery of the tumor, sunray-stream-like new bone formation was observed. This new bone formation was found in the extracortical portion of the tumor. Furthermore, histologically, sarcomatous cell was never seen in this area as shown in Fig. 20. This suggests that the extracortical new bone is of periosteal origin.

Intramedullary implantation of HORIE's bone sarcoma was also performed by the author in rats of the Wistar strain. HORIE's bone sarcoma has such histologic characters as spindle-shaped stromal cells, atypism of cells and cell nuclei, bizarre nuclei, mitotic division, and multinuclear giant cells (Fig. 21). When HORIE's sarcoma was implanted into the tibia, it caused more extensive new bone formation than the author's methylcholanthrene fibrosarcoma. Roentgenographic examination revealed bulging of the cortex, radiolucent areas, and extensive periosteal new bones at two weeks after the implantation (Fig. 22). By

histologic examination, sarcomatous cells were never found in the region of new bone.

V. SUBPERIOSTEAL TRANSPLANTATION OF EXPERIMENTALLY PRODUCED FIBROSARCOMA OF BONE

MATERIAL AND METHOD

The experimentally produced fibrosarcoma of bone was implanted beneath the periosteum in 73 young rats of the same strain (Gifu-Donryu strain, weighing 80-100 g). The tumor tissue was ground in a steril glass crusher and then homogenized in normal saline. A small quantity of the suspension (0.05 ml) was injected beneath the periosteum of the left tibia (Fig. 23). This method of injection is simple, however, some technical skill is necessary for its performance. Tumor bearing animals died 11 to 49 days after the implantation, with an average of 30.0 days. All animals were autopsied at death or sacrificed and preserved in 10 per cent formalin solution.

RESULTS

The results of subperiosteal implantation of fibrosarcoma are summarized in Table 7. In 38 animals tumor developed in the left leg and, in addition, in 16 of them metastases appeared in the lung. The rate of successful transplantation was 76.0 per cent and the rate of metastasis to the lung was 42.1 per cent. These results were compared with those of intramedullary transplantation in Table 8. The rate of metastasis was remarkably higher in cases of subperiosteal transplantation than in cases of intramedullary transplantation.

Table 7 Results of Subperiosteal Transplantation of Fibrosarcoma of Bone Induced by 20-Methylcholanthrene in Case 207.

Nos. of Rats	Invalid	Valid		Rate of Transpl. (per cent)	Rate of Metast. (per cent)	Period 'till Death of the Animal after the Transplantation (days)	
		Takes	Neg.			Min.-Max.	Average
73	13	38	22	76.0	42.1	11-49	30.0

Table 8 Difference of Rates of Successful Transplantation and Metastasis due to Sites of Implantation

Site of Implantation	Rate of Successful Transplantation (per cent)	Rate of Metastasis (per cent)
Bone Marrow	68.6	18.0
Subperiosteal Space	76.0	42.1

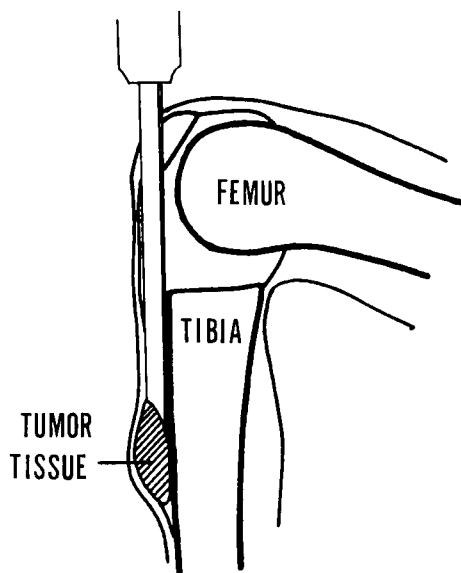


Fig. 23. Technique of subperiosteal implantation of the tumor.



Fig. 24. Roentgenogram of the tibia taken at four weeks after the implantation. The tumor invaded the knee joint and the femoral condyles were dissolved.



Fig. 25. Roentgenogram of the tibia taken at four weeks after the implantation of the tumor. Pathologic fracture occurred in the shaft.

Development of bone tumor beneath the periosteum of the tibia.

One week after the implantation swelling was noted in the left leg by palpation. The tumor grew rapidly and attained the size of $3.0 \times 3.0 \times 3.0$ cm within three weeks after the injection.

Roentgenographic findings:

The tibia seemed to be normal for one week after the inoculation. At the end of third week, decalcification, rarefaction of bone structure, and erosion of the cortex were found. Four weeks after the injection, bone destruction was observed in the shaft and the proximal metaphysis of the tibia (Fig. 24). Pathologic fracture of the tibia was seen in some cases (Fig. 25).

Microscopic findings:

One week after the implantation tumor cells beneath the periosteum did not show rapid growth. Within two weeks, the tibia was surrounded by the proliferated tumor tissue. Three weeks after the implantation, both the tibia and the fibula were embedded in the tumor tissue and the cortex became irregular, eroded, thin, and atrophic (Figs. 26 and 27). Spicules of new bone were already laid down in the tumor tissue, particularly at the periphery where the periosteum was elevated by the advance of the tumor tissue under it. The new bone was seen in radiating or lamellar arrangement outside of the cortex (Figs. 28, 29 and 30). Three or four weeks after the implantation, the process of destruction remarkably advanced, occasionally breaking into the joint cavity.

VI. DISCUSSION

Bone tumors were experimentally induced by 20-methylcholanthrene in the femur of 11 rats. The tumors were fibrosarcomas of moderate malignancy except for one osteogenic sarcoma. As already mentioned, several investigators have reported the experimental production of bone sarcomas with 20-methylcholanthrene (Table 9). These sarcomas showed many varieties of histologic features. In the author's study the tumors induced were central

Table 9 Reports on Bone Sarcoma induced by 20-Methylcholanthrene.

Author	Animal used	Period needed for production of palpable tumor	Rate of tumor production (per cent)	Histologic findings
Brunschwig (1938)	Rat	8-12 Mos.	12.1	Fibrosarcoma
Franseen et al. (1941)	Mouse	91-225 Days	22.9	Fibrosarcoma
Obayashi (1959)	Rat	9-43 Weeks	28.7	Fibrosarcoma, polymorphous cell sarcoma, and spindle cell sarcoma
Makita (1960)	Rat	247-410 Days	50.0	Osteogenic sarcoma, fibrosarcoma, reticulum cell sarcoma, and polymorphous cell sarcoma
Chino (1960)	Rat	12-24 Weeks	30.0	Fibrosarcoma and spindle cell sarcoma
Yamada (1963)	Mouse	78-298 Days	13.6	Bone forming sarcoma, fibrosarcoma, spindle cell sarcoma, and giant cell tumor
The author (1966)	Rat	22-47 Weeks	33.3	Osteogenic sarcoma and fibrosarcoma

and parosteal in origin. One of the fibrosarcomas, which has been successively transplanted into the subcutaneous tissue of rats was capsulated with a thin fibrous membrane and showed no evidence of metastasis. The cut surface of the tumor was grayish-white at the central part and reddish-gray, soft, and friable at the peripheral part. Histologic study revealed that it was fibrosarcoma with malignant features such as lack of uniformity of cells and cell nuclei, hyperchromatic and bizarre nuclei, and mitotic division. During the course of successive subcutaneous transplantation over 16 generations for one year, these gross pathologic and histologic figures remained unchanged.

This fibrosarcoma of bone was implanted into the bone marrow and beneath the periosteum of rats of the same strain.

When the tumor was implanted into the marrow cavity of the tibia, there was a high percentage (68.6 per cent) of takes. Furthermore, metastasis also frequently appeared in various organs (23.1 per cent). The tibia exhibited destruction of trabeculae, bulging of the cortex, and tumor growth beneath the periosteum in two weeks after the implantation. Metastasis occurred in the lung in 11 of 61 host animals (18.0 per cent) in which tumor developed in the tibia. Metastasis appeared in the liver in two cases and in the spleen in one case.

Implantation of the tumor beneath the periosteum of the tibia also showed a high incidence of tumor take (76.0 per cent) and metastasis (42.1 per cent). Transferred tumor tissue showed palpable enlargement in one week, and bone destruction occurred three weeks later.

In these two experiments spicules of new bone were found in the tumor tissue, especially at the peripheral parts where the periosteum was elevated by invaded tumor tissue. The metastatic lesion grew in the cytologic pattern of the original tumor.

The following conclusions were obtained from the results of this series of experiments on the tumor induced by 20-methylcholanthrene.

1. All the tumors induced in this series of experiments were fibrosarcomas of poor differentiation, except for one osteogenic sarcoma.

2. When fibrosarcoma was transplanted in the bone marrow and beneath the periosteum of rats of the same strain, a high rate of tumor take was proven.

3. Bone destruction occurred earlier in the cases of intramedullary transplantation than in the cases of subperiosteal transplantation.

4. Metastasis occurred in both series of experiments on intramedullary and subperiosteal transplantation. However, the rate of metastasis was higher in the latter than in the former.

5. Spicules of new bone were also found in both series. However, new bone formation in the tumor occurred at a higher rate in the group of subperiosteal transplantation than in another group.

6. The results obtained from these experiments on this transplantable fibrosarcoma of bone are thought to be useful materials on which the biologic behavior of bone sarcoma can be analyzed.

Carcinogenic Mechanism of Chemical Excitants.

Many attempts have been made to explain the carcinogenic mechanism of chemical excitants^{35,49,54,64}. From the phenomenologic standpoint of view, BERENBLUM introduced the two-stage hypothesis on carcinogenesis, the concept of dormant cells induced by initiating action and their conversion into growing tumors by promoting action. He suggested that if the initiating action converts normal cells into dormant tumor cells and this promoting action causes these dormant tumor cells to develop into visible tumors, the number of tumors produced would be determined by the potency of the initiating process, and the speed with which they grew would be dependent on the efficacy of the promoting process^{4,5}. NAKAHARA and FUKUOKA suggested that a summation of the actions of various carcinogens play a role in the tumor development⁴⁴. However, none of these hypotheses explain the mechanism of carcinogenesis entirely. The causes of cancer have still remained obscure^{15,31,63,65,66,76}.

Is the Tumor which was Induced by 20-Methylcholanthrene Central or Parosteal in Origin ?

FRANSEEN et al. performed an experimental study on the production of fibrosarcomas of bone by 20-methylcholanthrene and stated that these fibrosarcomas were of parosteal origin. They assumed that this neoplasm may originate from a small area of fibroblastic repair tissue in the drill hole in the bone in which the methylcholanthrene was inserted¹⁹. CHINO who studied the production of bone tumor by the intramedullary injection of methylcholanthrene assumed that the tumor induced by this agent was of extra-osseous origin¹³.

On the other hand, BRUNSWIG, BRUNSWIG and BISSELL, and the author have shown that central fibrosarcoma or osteogenic sarcoma may occur by the intramedullary administration of methylcholanthrene^{11,12}. OBAYASHI injected carbon powder into the bone marrow of the femur of the rat and observed that the greater part of it remained within the bone marrow and only a small quantity of it reached beneath the periosteum through VOLK-

MANN's canal. He assumed that intramedullary injected methylcholanthrene may also reside within the bone marrow and beneath the periosteum and induce bone sarcoma of both central and parosteal origin⁴⁶⁾. BRUNSCHWIG claimed that fibrosarcoma induced by methylcholanthrene are either parosteal or central in origin. The roentgenographic and histologic appearances of experimentally induced fibrosarcoma of the central type were identical in character with central fibrosarcoma observed in man. BRUNSCHWIG and BISSELL reported an osteosarcoma (bone-forming sarcoma) of the tibia of a mouse induced by an intramedullary implantation of a mixture of 1,2-benzpyrene and cholesterol. In the author's investigation, two of 11 tumors were central in origin. Roentgenographic, gross pathologic, and histologic studies revealed that one of them was osteogenic sarcoma (central osteogenic sarcoma) and the other was central fibrosarcoma.

Why is Fibrosarcoma More Likely Induced by Methylcholanthrene Than Osteogenic Sarcoma ?

As shown in this experiment, it is clear that fibrosarcoma of bone is more likely induced by methylcholanthrene than is osteogenic sarcoma. BRUNSCHWIG presumed that the number of fibroblasts in bone which does not function as osteoblastic is much in excess of the number of active and potential osteoblasts and the carcinogenic agent, therefore, is in contact with a relatively greater number of the former. FRANSEEN et al. expressed the view that young fibroblasts are responsible for the tumor development. SCHÜRCH and UEHLINGER revealed that through the intramedullary application of radium or mesothorium, four osteogenic sarcomas, one EWING's sarcoma, one extraosseous, fibrosarcoma, and three central and periosteal spindle cell sarcomas were produced^{60,62)}. Another experiment of SCHÜRCH and UEHLINGER showed that five osteogenic sarcomas were produced by the intramedullary application of radium and mesothorium⁶¹⁾. MAKITA reported the four osteogenic sarcomas, one fibrosarcoma, one polymorphous cell sarcoma, one reticulum cell sarcoma, and one rhabdomyosarcoma induced by intramedullary injection of 20-methylcholanthrene combined with simultaneous administration of radioactive phosphorus³⁹⁾. YAMADA reported that, out of 15 bone sarcomas of mice induced by methylcholanthrene, one was osteogenic sarcoma⁷⁹⁾. In the author's experiment, one osteogenic sarcoma and ten fibrosarcomas were produced in the femur of rats.

As many investigators have shown, when beryllium is injected intravenously, the deposition occurs selectively in bone. Though beryllium acts upon fibroblasts as well as upon osteoblasts, this agent induces the tumor of osteogenic or chondroblastic origin with extremely high frequency. In BETZLER's study, 61 fibrosarcomas were produced in 49 rats by subcutaneous or intramuscular injection of methylcholanthrene⁷⁾.

These facts suggest high susceptibility of fibroblasts to this agent. In general, it can be said that in order for sarcoma to develop there must be a susceptible tissue and an inciting agent. The fibroblasts are most susceptible to the carcinogenic action of 20-methylcholanthrene and the osteoblasts and chondroblasts are most susceptible to the carcinogenic action of radioactive substances and beryllium. From this point of view, the fact that, in the author's experiment, the fibrosarcomas were produced with high frequency seems to be well explained by the high susceptibility of fibroblasts to 20-methylcholanthrene.

The New Bone Formation in the Tumor.

There are only a few reports in the literature on subperiosteal transplantation of the experimental bone sarcoma. In the author's experiment of subperiosteal transplantation the new bone formation and the metastasis to the lung were conspicuous findings. Also in the case of intramedullary transplantation, new bone has occurred in the areas where the tumor tissue has destroyed the bone cortex and invaded beneath the periosteum. However, more frequent and more intense occurrence of the new bone formation was demonstrated in the study of transplantation beneath the periosteum. These facts are considered to be helpful for the elucidation of osteogenesis in bone sarcoma in man.

BRUNSCHWIG has shown that transplantation of epidermoid carcinoma into the bone marrow cavity produced spicules of new bone as in osteogenic sarcoma¹⁰). MIYAGI and TANIGUCHI observed the formation of spicule-like new bone tissue in the intramedullary transferred HORIE's sarcoma^{40,47}). TANIGUCHI also found osseous tissues in the metastatic tumor in the lung. However, as he suggested, these osseous tissues found in the pulmonary metastatic tumor were supposed to be non-tumorous. In the author's study, HORIE's sarcoma did not show any ossifying tendency in the metastatic pulmonary tumor. IKEDA, KUZUOKA and others showed that transplantation of WALKER's carcinosarcoma 256 into the bone marrow induced a sunray-stream of new bone^{25,30,34,47}). CONNER showed in his study of transplantation of bone sarcoma that the tumor cells formed radiating spicules of bone, while the tumor was confined between the periosteum and the cortex¹⁴). As WEPHWADZE suggested, when BROWN-PEARCE carcinoma was inoculated subperiosteally and into the bone marrow, typical spicules were seen, as in the sclerosing sarcomas⁷⁷). These spicules reported by many investigators are presumably produced by an osteogenic element of the periosteum.

PHEMISTER expressed the view that two types of new bone may be formed, namely tumorous and non-tumorous, and it is usually possible to tell them apart in the roentgenograms. Tumorous bone, either in the primary lesion or in the metastatic lesion, arises from tumor cells which possess an inherent tendency to ossify. Non-tumorous bone arises from osteoblasts of the normal bone in which the tumor develops. Non-tumorous new bone may arise either from the periosteum or the endosteum in the vicinity of the tumor. The new bone is of a spongy structure and very similar to the callus formed in healing fractures, resembling also to the new bone formed in osteomyelitis. Non-tumorous new bone may grow from the old cortex into the substance of the tumor and form a supporting framework, which radiates similarly to tumorous new bone in ossifying sarcoma. In this case it may be difficult to distinguish it from tumorous new bone⁵³).

Spicules of new bone may be formed by osteogenic elements of the normal bone in which the experimental tumor is implanted. This new bone exhibits frequently similar roentgenographic and histologic features as those which are found in osteogenic sarcoma in man. Nevertheless, when the original experimental tumor cells do not possess the capacity of ossification, the new bone is thought to be non-tumorous.

In the author's experiments trabeculae of new bone were formed in the extracortical portion of the tumor, growing out from the old cortex in the substance of the tumor and showing radiating or lamelled arrangement of bone trabeculae in which tumor cells were

never seen. Moreover, the original tumor cells have never presented any evidence of neoplastic osteoid and osseous tissue in the course of subcutaneous transplantation over sixteen generations for one year. From these facts, the original tumor is defined as a specialized connective-tissue sarcoma which never forms neoplastic osteoid and osseous tissue in the course of evolution and the new bones formed in the tumor tissue during the course of the transplantation experiments is supposed to be non-tumorous.

Metastasis

Metastasis is one of the most characteristic signs of the malignant tumor and the malignancy of experimental tumor is generally accepted when the tumor metastasizes to other organs or tissues. Tumor cells in the metastatic lesion are equivalent to the cells of the primary lesion in regard to composition of genes, cytologic features, histologic findings, and the mode of evolution. The histologic features of the metastatic lesion is, nevertheless, occasionally modified by the local stromal responses⁵²⁾.

There is a general agreement about the factors which influence the establishment of metastatic lesions. They are; 1) infiltrating and destructive growth of primary tumor tissue, 2) release of the tumor cells from the primary lesion, 3) invasion of the tumor cells into vessels, 4) active movement of the tumor cells in the vessels, 5) arrival and settlement of the tumor cells in other organs or tissues, and 6) development of the tumor cells in the other organs or tissues. The metastatic evolution may also be modified by the function of the reticuloendothelial system, the nutritive and circulating conditions, and the numbers of the tumor cells which proceed into the metastatic region^{50, 51, 59)}.

Occasionally metastasis is established in an extraordinary way of occurrence. Solitary metastasis or special affinity between tumor cells and the host organ (e. g. carcinoma of the prostate preferably metastasize to the bone) is not uncommon²⁷⁾. The solitary metastasis and the predilection of metastatic lesion cannot be explained by the circulating conditions or the numbers of the tumor cells which invade the blood stream^{28, 32, 37, 38, 41, 55, 69, 73)}. Also there is no general agreement about the relationship between the primary tumor and the metastatic tumor concerning the size and number. Moreover, it is a well known fact that intravenous injection of tumor cells results not always in metastasis to the lung^{58, 72, 80)}. ZEIDMAN et al. were unable to establish a significant correlation between the size of the primary tumor and the number of lung metastasis⁸¹⁾. WOOD et al., on the contrary, suggested that there was a positive correlation between the final size attained by the primary tumor in a given time and the number of lung metastases⁷⁸⁾.

In the author's experiments two modes of transplantation were employed: transplantation of sarcoma into bone marrow and beneath the periosteum. High occurrence of metastasis was expected in the intramedullary transplantation of sarcoma, because a considerable number of the tumor cells would be brought into the blood stream immediately after the transplantation. However, a higher incidence of metastasis was found, not in the transplantation into the bone marrow, but in the transplantation beneath the periosteum. The only possible explanation for such an unexpected result is presumably that the development of metastasis may not occur immediately after the implantation and that in case of subperiosteal implantation the tumor invades into the surrounding soft tissue, resulting in

rapid growth of the tumor followed by metastasis.

SUMMARY

1. Experimental production of bone sarcoma was attempted and 11 tumors (one osteogenic sarcoma and ten fibrosarcomas) developed in 33 rats.

2. One of the fibrosarcomas has been successively transplanted in the subcutaneous tissue of rats of the same strain. Histologic examination revealed that the tumor was fibrosarcoma with poor differentiation. During the successive transplantation over sixteen generations for one year, gross pathologic and histologic features of the tumor remained unchanged.

3. Transplantation of the fibrosarcoma into the bone marrow and beneath the periosteum was performed. A high rate of tumor take was proven in both series of experiments.

4. New bone formation in the tumor tissue and the metastasis to the lung were conspicuous findings of the transplanted tumor in both series of experiments. However, a higher incidence of new bone formation and metastasis was found in the cases of transplantation beneath the periosteum than in the cases of intramedullary transplantation.

5. The new bone formed in the course of transplantation experiments is considered to be non-tumorous.

6. This kind of fibrosarcoma is presumed conclusively to be one of the useful materials for the experimental study of bone sarcoma.

7. Discussion was presented concerning the origin of the experimentally produced bone sarcoma and the biologic mechanism responsible for new bone formation in the tumor tissue and metastasis.

The author is deeply indebted to Prof. Dr. TETSUO ITO, Emerit Prof. Dr. EISHI KONDO, and Assoc. Prof. Dr. YOSHIHIKO AKAHOSHI for their kind advice and encouragement throughout this study. At the same time the author is grateful to Dr. SADAYOSHI TANIGUCHI and Mr. RYOICHI KASAI for their kind assistance.

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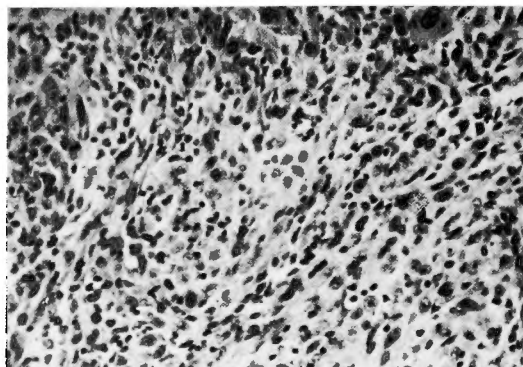


Fig. 4. Case 207. Hematoxylin and eosin, $\times 200$.

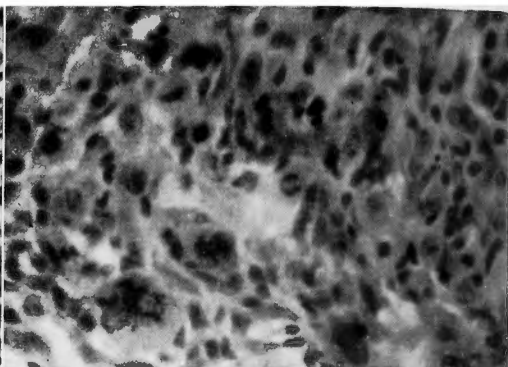


Fig. 5. Case 207. Hematoxylin and eosin, $\times 400$.

Figs. 4 and 5. Photomicrographs of the tumor in Case 207. Lack of uniformity in the cells and cell nuclei, spindle-shaped stromal cells, sarcomatous giant cells, and lack of osteogenesis.

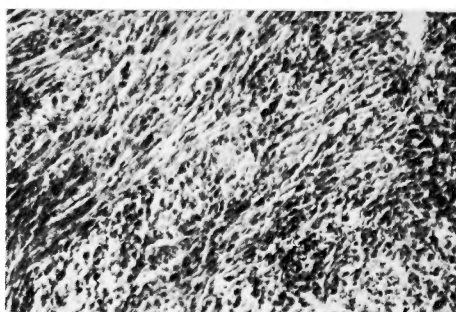


Fig. 7. Photomicrograph of the tumor in Case 204. The stromal cells are spindle-shaped and highly anaplastic in an unossified region. Hematoxylin and eosin, $\times 100$.

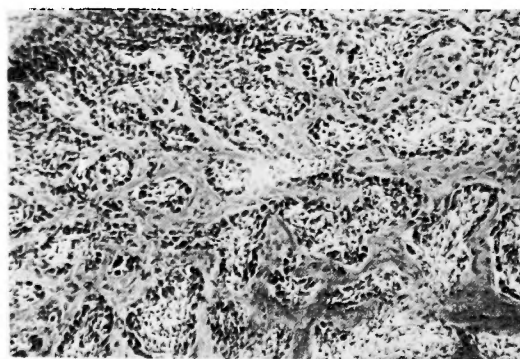


Fig. 8. Case 204. Hematoxylin and eosin, $\times 100$.

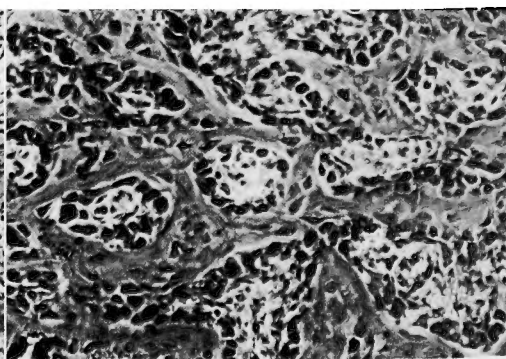


Fig. 9. Case 201. Hematoxylin and eosin, $\times 200$.

Figs. 8 and 9. Photomicrographs of the tumor in Case 204. New bone formation in the tumor. These new bone consist of loosely woven bone trabeculae interspersed with loose-meshed tumor cells which show evidence of moderate malignancy.

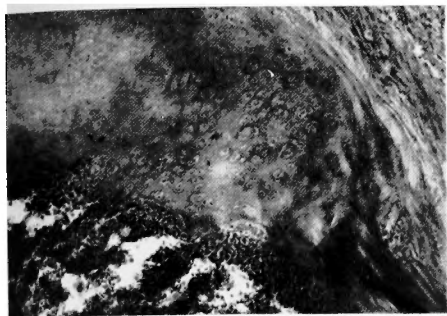


Fig. 10. Photomicrograph of the tumor in Case 204. Sarcomatous cells are differentiated in the direction of cartilage cells. Hematoxylin and eosin, $\times 50$.



Fig. 16. Photograph of a microscopic specimen of the tibia two weeks after the implantation. Note the destruction of cortical bone and development of the tumor tissue beneath the periosteum (\rightarrow). Hematoxylin and eosin, $\times 2$.

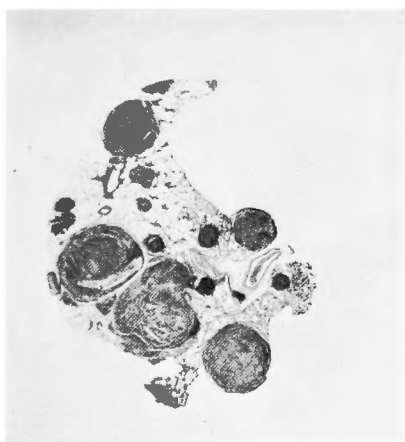


Fig. 17. Photograph of a microscopic specimen of the metastases to the lung. Hematoxylin and eosin, $\times 4$.

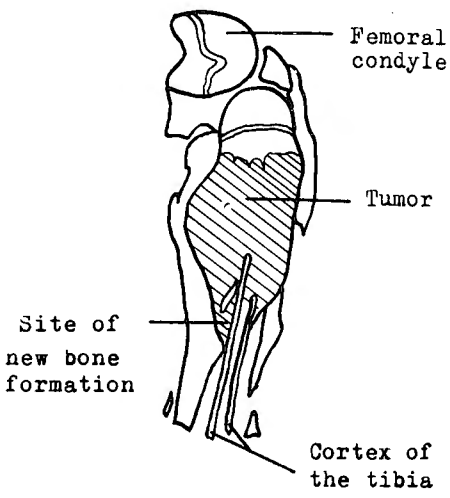


Fig. 19. Photograph of a microscopic specimen of the tibia after three weeks. Hematoxylin and eosin, $\times 2$.



Fig. 20. Photomicrograph of sunray-stream-like new bone formation in the tumor tissue. Hematoxylin and eosin, $\times 100$.

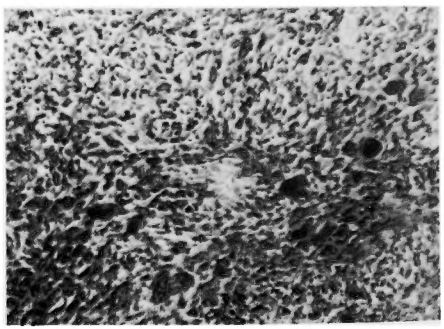


Fig. 21. Photomicrograph of Horie's bone sarcoma. Hematoxylin and eosin, $\times 100$.



Fig. 22. Roentgenogram of the tibia made at two weeks after the intramedullary implantation of Horie's sarcoma. Note the extensive periosteal new bones.

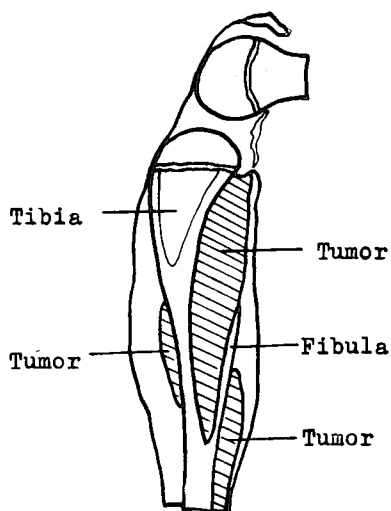
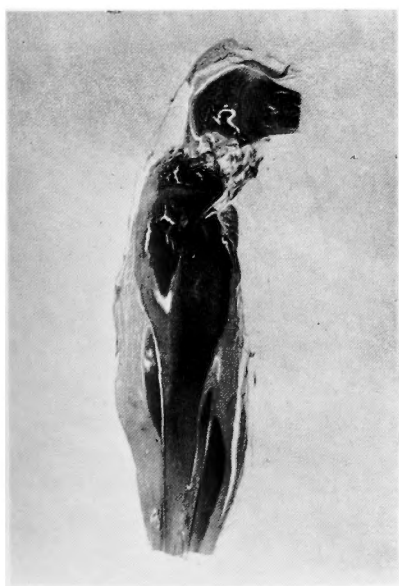


Fig. 26. Photograph of a microscopic specimen of the tibia taken at three weeks after the implantation. Both tibia and fibula are embedded in the tumor tissue. Hematoxylin and eosin, $\times 2$.

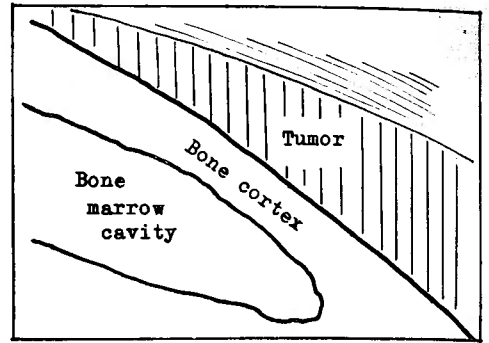
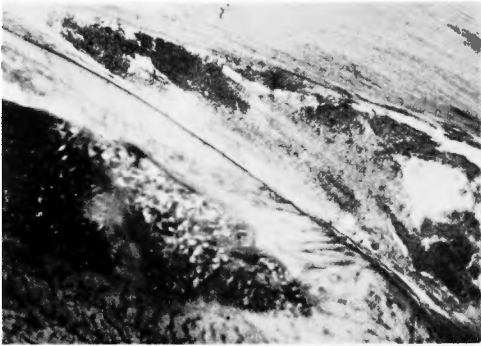


Fig. 27. Photomicrograph of the tibia taken three weeks after the implantation. The cortex is eroded, thin, and atrophic. Hematoxylin and eosin, $\times 20$.



Fig. 28. $\times 50$.



Fig. 29. $\times 100$.

Figs. 28 and 29. Photomicrographs of the new bones growing out from the old cortex in radiating arrangement. Hematoxylin and eosin.



Fig. 30. Photomicrograph of the new bones in laminated arrangement. Hematoxylin and eosin, $\times 200$.

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(* Written in Japanese.)

和文抄録

骨腫瘍の実験的研究

—20-Methylcholanthrene 骨髄内注入により発生せしめた骨腫瘍の病理—

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渡 辺 良

発癌性物質を用いて実験的悪性骨腫瘍を作製する試みは既に数多く行なわれているが、現在迄に作製された骨腫瘍は種々の点で人の悪性骨腫瘍とは異つて居り、人の骨肉腫にみられる様な、レ線像組織像における多様性を示す実験腫瘍は未だ報告されていない。著者は多核芳香環炭化水素 20-Methylcholanthrene の骨髄内注入により Gifu-Donryu 系ラッテに移植可能な実験骨腫瘍を作製し、これを用いて種々の移植実験を行なつた。

1) 33匹のラッテの内11匹に骨腫瘍の発生を認めた(骨肉腫1, 線維肉腫10)。腫瘍発現に要した期間は22週から47週に亘り、平均35週であつた。11例の腫瘍のうち2例(骨肉腫, 線維肉腫1)は central origin, 9例(線維肉腫)は parosteal origin のものと考えられる。

2) 線維肉腫の1例において継代皮下移植に成功した。本腫瘍は組織学的には未分化の線維肉腫で腫瘍性類骨組織又は骨組織を形成せず、1年間16代に亘る継代移植によつてその組織学的特性は変化しなかつた。移植陽性率は87.7%であつた。

3) 継代移植に成功した線維肉腫を同種ラッテの脛骨骨髓内および骨膜下に移植した所、移植陽性率は骨髓内移植68.6%, 骨膜下移植76.0%であつた。何れの

移植法においても腫瘍内の spicula 様骨新生と肺転移を認めたが、骨膜下移植の場合の方が骨髓内移植の場合よりも、腫瘍内骨新生、肺転移共に高率に認められた。肺転移率は骨髓内移植では18.0%, 骨膜下移植では42.1%であつた。本移植実験において認められた spicula 様骨新生は腫瘍細胞自体の骨形成によるものではなく、腫瘍発育によつて剝離された骨膜に主として由来するものと思われる。何故なら本移植実験に用いられた腫瘍細胞はその増殖の過程において腫瘍性類骨組織又は骨組織を形成せず、従つて腫瘍細胞自体に骨形成能はないと考えられるからである。本実験において観察された骨新生は人の骨腫瘍における骨新生あるいは spicula の本態を解明するための手がかりのひとつとなり得るものと思われる。

4) 著者の作製した線維肉腫は、移植率が大であること、移植方法を考慮することにより肺転移率も高くなる可能性があること、更に移植操作を一定にすることにより動物の生存日数や局所腫瘍の発育度をほぼ一定にし得ると思われること、などにより実験骨腫瘍の有用な株のひとつとして使用しうると考える。

(尚、本論文の要旨は第95回近畿外科学会において発表した)。